Front. Biol. 2014, 9(2): 161–167
DOI 10.1007/s11515-014-1296-0

RESEARCH ARTICLE

Microbial diversity in coastal sediments during pre- and post-tsunami periods in the south east coast of India

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Abstract **Sediment samples were collected from 12 beaches affected by the 2004 Asian Tsunami in the south-east coast of India between Vanagiri and Nagoor. The objective of the present study is to delineate the microbial diversity in pre- and post-tsunami disaster coastal sediments. The collected marine sediments indicate that the overall microbial diversity is higher in the pre-tsunami sediments. The increase in pathogenic bacteria and fungal species after the tsunami is obscured due to inundation and backwashing of seawater along the coast. The reduction of other microbial diversity after the tsunami is attributed that the coastal and shelf sediments play an important role in the demineralization of organic matter, which supports the growth of microbes. The continuous exchange of ocean water and backwashing of coastal sediments by the tsunami wave probably reduced the pathogenic bacterial diversity in the sediments.**

Keywords bacterial diversity, tsunami, coastal sediments, fungal diversity, principal component analysis, person correlation matrix

Introduction

Tsunamis are sea surface gravity waves generated by large-scale underwater disturbances. Typical trigger mechanisms are earthquake initiated seabed displacements, volcanic eruptions, landslides (including underwater landslides), impact of large objects (such as meteors) falling into the open ocean and underwater explosions. These waves are known to be able to cause significant alterations in the coastal systems (Dawson, 1994; Bryant et al., 1996; Bryant, 2001; Scheffers and Kelletat, 2003). They may produce extensive changes in coastline topography, considerable erosion and subsequent deposition of substantial quantity of sediments in a short span. The December 2004 Asian earthquake and the subsequent tsunami off the east coast of Sumatra in Indonesian Archipelago have affected most of the countries around the Indian Ocean. The tsunami deposits in the land along the south-east coast of India have signatures of different geological features, which inundated to deeper regions (Nagendra et al., 2005; Srinivasalu et al., 2007, 2008). Many studies are available on ancient and recent tsunami deposits, including the descriptions of tsunami deposits in coastal lake, estuary, lagoon, bay floor, shelf and deep sea tsunami deposit environments (Konno, 1961; Bourgeois et al., 1988; Yamazaki et al., 1989; Cita and Aloisi, 2000). In general, disastrous tsunamis change the biodiversity of marine ecosystems, because the tsunami waves travel thousands of kilometers in the sea, with a lot of washed out materials from the land. One of the most significant phenomena related to tsunami inundations is large-scale sediment removal from the coast, followed by widespread deposition of marine sand on coastal lowlands (Minoura and Nakaya, 1991). So there will be a possibility of demineralization in the marine environment. The impact of tsunami spreading their traces not only to the life of marine benthos and also it affects the diversity of marine microbes (Altaff et al., 2005).

Historical evidences revealed that, only two earth quakes (1881 and 1941) have made a mysterious tsunami effect in the east coast of India (Rajendran et al., 2005). The marine ecosystem is affected by physical disturbances like tsunami, hurricanes and storms etc. which affect the benthos by changing the sediment characteristics and available food

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Received September 15, 2013; accepted February 7, 2014

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resources (Boesch et al., 1976a; Van Blaricom, 1982; Thistle et al., 1995). Several researchers have undertaken studies related to the impact of tsunami on microbial population in south-east coast of India (Subramani et al., 2006; Ramesh et al., 2006; Mahalakshmi et al., 2011; Surajit Das et al., 2013). When giant tidal waves like the tsunami occur in the sea, it is possible that the deeper nutrient rich water comes up to the surface (Levinton, 2001). This might have influenced the marine environment to a great extent because of nutrient and mineral flux resulting in variation in marine biodiversity in pre- and post-tsunami sediments. This has prompted to assess the microbial diversity in pre- and post-tsunami sediments in the study area. Microorganisms also have an important and established biogeochemical role within intertidal ecosystems, carrying out crucial transformations and remineralization of organic matter (Decho et al., 2005). Most of the microbes like *Vibrios, Halomonas, Pseudomonas* sp. *and E. coli* are pathogenic to human health and some lead to fatal infectious (Carlson et al., 1968; Grimes, 1975; Gerba and Schaiberger, 1975; Blacke et al., 1981). The diversity, occurrence and activity of heterotrophic bacteria are controlled by several hydro biological factors and nutrient levels present in the aquatic environment and have been well established in marine ecosystem (Azam et al., 1983; Ducklow and Hill, 1985). The environmental constraints like high temperature, exposed to UV (ultra violet) radiation, drought, pysico-chemical alterations with intense marine biological interactions create a competition for food and space to the microbial population (Lindow and Brandl, 2003). The population density of *Vibrio* sp. in the marine environment is usually high because it can withstand a wide range of aquatic environments including estuaries and marine sediments (Urakawa et al., 2000; Thompson et al., 2004). Microorganisms spreading in the marine and brackish ecosystem play an important role in the decomposition of organic nutrients and enhance mineralization (Hollibaugh et al., 1980). Hence there has been an endeavor to evaluate the impact of tsunami on microbial diversity.

Study area

The study area extends to about 35 km between Vanagiri and Nagoor (11°08′47.1″ to 10°48′80.7″ N and 79°51′42.6″ to 79° 51′05.8″ E) in the south-east coast of India (Table 1). It is located north of Point Calimere region at the southern tip of the Bay of Bengal. The rivers Uppanar, Vellar, Gadilum and Cauvery pass through the granitic terrain and agricultural belt in Tamil Nadu State before draining into the Bay of Bengal. The coastal stretch is affected by the seasonal monsoon (every year) in the latter half of the year (October–December) (Sarma et al., 1990). The study area is normally shallow, with the depth varying from 5 to 30 m for nearly 80 km^2 parallel to the coast and the shallow nature extends into the Palk Strait region in the southern side (Stephen-Pichaimani et al., 2008). The geology around the study area indicates different types of rocks which include alluvium, charnockite, khondalite, garnet-sillimanaite gneiss, pink/gray granite, amphibolites, pyroxenites and biotite schists, which lie in the southern part of the study area (Vasudevan and Seetaramaswamy, 1983). In addition, the southern part of Cauvery basin (part of Nagapattinam and Karikal Town) in the peninsular shield is underlain by rocks of Archean age and the coastal tract is covered by younger alluvium and coastal sands (Mohanachandran and Subramanian, 1990). The study area also witnessed maximum backwash of sediments from land during/after the three major tsunami waves that struck the coastal region (Srinivasalu et al., 2009).

Materials and methods

Intertidal Marine sediments (0–15 cm) were collected from 12 sampling locations along the south-east coast of India during pre- and post-tsunami periods. The sediments were collected using a handy sampler in sterile polypropylene bags and transferred to laboratory for further analysis. The wet marine sediments were sub-sampled for isolation of cultured bacteria

Table 1 Geographical locations of the study area

Sl. No.	Station code	Station name	Latitude (N)	Longitude (E)
1	**S1**	Vanagiri	11°07.703′	79°51.476′
2	**S2**	Chinnankudi	11°05.429′	79°51.437′
3	**S3**	Vellakoil	11° 03.186′	79°51.342′
4	**S4**	Tharangampadi Fort	11°01.452′	79°51.326′
5	**S5**	Chandrapadi	10°59.754′	79°51.274′
6	**S6**	Akkampettai	10°58.199′	79°51.218′
7	**S7**	Kottucherry medu	10°57.710′	79° 51.210′
8	**S8**	Kasavakudi	10°56.986′	79°51.188′
9	**S9**	Karaikkal Beach	10°54.915′	79°51.140′
10	**S10**	Pattinamcherry	10°53.113′	79°51.140′
11	**S11**	Vadakuvanjur South	10°50.800′	79°51.141′
12	**S12**	Nagoor Beach	10°48.807′	79°51.058′

and fungi. A serial dilution was performed by adding 1 g of soil to 99 ml of sterile distilled water and diluted serially to 10^{-6}. The plates were incubated at room temperature up to 48 h in the case of bacteria and 5 days for fungi. The microbial diversity was counted and expressed as colony forming units (CFUs) per gram of sediments. Individual colonies were picked up and subcultured in the respective medium, and the colony characteristics such as size, shape, pigmentation and exopolysaccharide (EPS) production, etc. were recorded. The bacteria isolated from pre- and post-tsunami sediments were categorized on the basis of Gram's reaction. All the isolated bacteria were streaked on Eosin Methylene Blue agar selective medium to determine the Coliforms (Vieira et al., 2001) and Zobell marine agar medium is used to culture other halophilic bacteria. The same Rose Bengal media can be used as its composition encourages the fungal diversity. *Vibrio* species were determined in Thiosulfate Citrate Bile-salt Sucrose agar and further confirmed by their shape through microscopic investigation (Arias et al., 1998). Bacterial diversity was determined by Paul and Clark (1998) method.

CFUs were calculated by the following formula

$$\mathrm{CFUs/mL} = \text{Number of colonies}/\text{dilution} \times \text{amount plated}$$

Results

The present investigation highlights the ubiquitous distribution of bacterial diversity in the marine sediments during the pre- and post-tsunami periods, along the south-east coast of India. Based on the colony morphology, 26 strains were selected and sub cultured, and 5 generas were identified viz. *Vibrio, Halomonos, Serratia, Pseudomonas* and *Escherichia*. Among these, *Vibrio* constituted 33.3% followed by *Serratia* (28.7%), *Escherichia coli* (14.3%), *Pseudomonas* (12.9%) and *Halomonos* (10.6%). All the isolated bacterial species belonged to the gram negative group. In the case of pathogenic bacteria, a total of 3 species (*Vibrio Parahemolyticus, Pseudomonas aeruginosa* and *Escherichia coli*) were recorded (Fig. 1).

The occurrence of more number of pathogenic species (bacteria and fungus) in the pre tsunami sediments of the study area maybe due to the confluence of rivers with urban runoff. The population of microbes and fungi species are drastically reduced and some of the pathogenic microbes are noticed after backwashing effect of the tsunami inundation. This may be due to the withstanding behaviour of such pathogens. They can survive beyond the extreme condition i.e tsunami effect.

Population density of bacterial species during pre- and post-tsunami periods in the coastal sediments varied from 14 × 10^6 to 25 × 10^6 CFU/g and 4 × 10^6 to 9 × 10^6 CFU/g. In general, higher bacterial diversity is due to rich organic matters and high residence time of the microorganisms in the sediments. The bacterial diversity is drastically reduced in tsunamigenic sediments except pathogenic species (Fig. 2). According to Surjit Das et al. (2005) the higher diversity of pathogenic bacteria species along the coast after the tsunami is most probably due to inundated tsunami water. The mean population density of pathogenic bacteria species during pre and post tsunami periods ranges from 4.0 × 10^6 to 7.4 × 10^6 CFU/g. Halophilic *Vibrios* can represent as much as 40% of the total microbiota of the subtropical coastal waters (Cheung et al., 1990). The considerable population density of *Vibrio* spp. (31.5%) in the pre- and post-tsunami, marine environment suggests that they survive in a wide range of aquatic environments including estuaries, marine, coastal waters and sediments. The low diversity of *Escherichia coli* in the study area indicates less pollution in the coastal sediments. Among the pathogenic species, no *Pseudomonas aeruginosa* diversity was recorded and a considerable population density of *Escherichia coli* and *Vibrio parahemolyticus* (25 × 10^6 CFU/g) was retained after the tsunami. The decrease of other bacterial diversity in the sediments after the tsunami is attributed to the demineralization of organic matter from the coastal and shelf sediments which support the growth of microbes. The continuous exchange of ocean water and

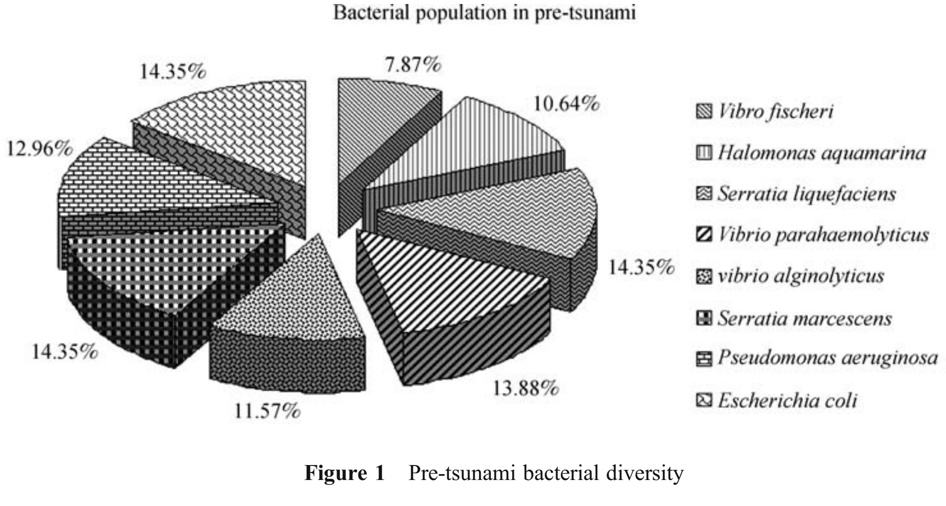

Figure 1 Pre-tsunami bacterial diversity

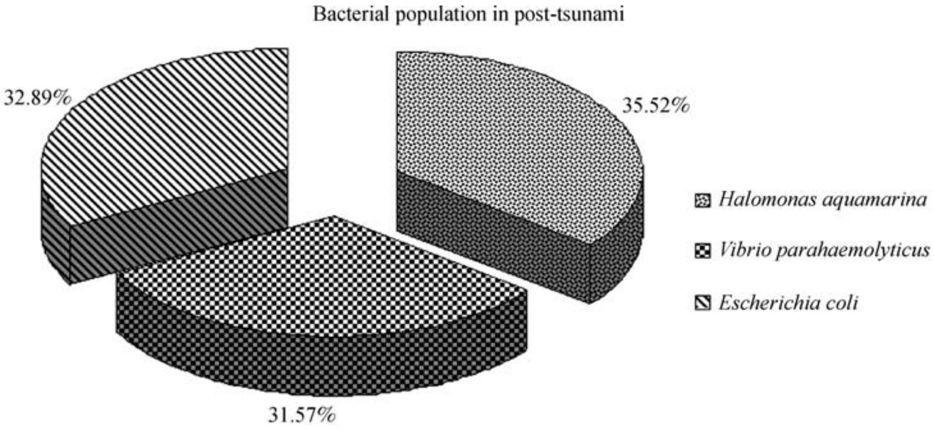

Figure 2 Post-tsunami bacterial diversity

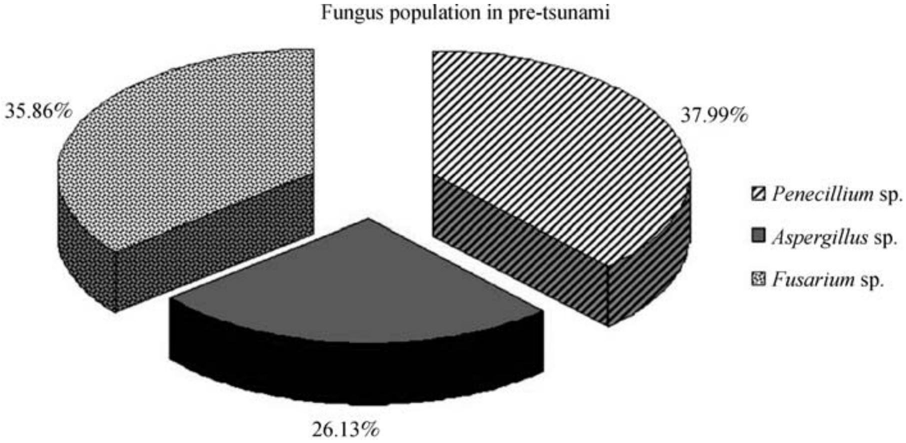

Figure 3 Pre-tsunami fungus diversity

backwashing of coastal sediments during the tsunami wave or the tidal wave or the tidal oscillation in the full moon and newmoon period might have reduced the pathogenic bacterial diversity in the sediments.

Fungal species such as *Penecillium, Aspergillus* and *Fusarium* were recorded in the study area (Fig. 4). Population densities of fungal species like *Penecillium* and *Aspergillus* were more in post tsunami sediments when compared to pre tsunami sediments (37.9% to 49.7% and 26.13% to 43.5%) (Fig. 3). It was also observed that *Fusarium* sps. in the post tsunami sediments were considerably decreased from 35.8 to 20.4%.

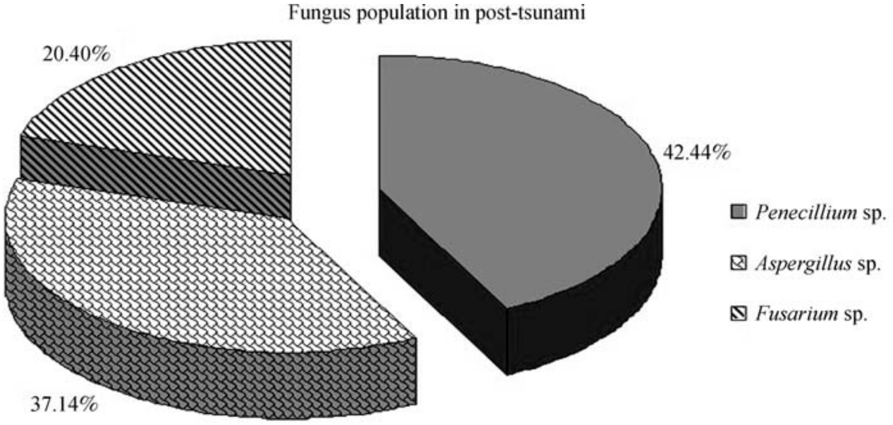

Figure 4 Post-tsunami fungus diversity

Pearson correlation matrix (PCM)

Pearson correlation matrix was used to determine the relationship among the sediment, nutrients with the physico-chemical properties and microbial diversity of the marine sediments and to estimate the relation between the variables. Correlation matrix was calculated among all the four variables for different locations in both pre- and post-tsunami sediments and a comprehensive chart is prepared in Table 2 A and 2B.

A good correlation has been observed between amino acid and carbohydrate in pre-tsunami. However, after tsunami the correlation grows negatively. It shows slightly inversely proportional between these two parameters. Moreover, a negative correlation ($r = -0.509$) has observed between bacteria and carbohydrate in post-tsunami period. However, it is noticed that there is no relationship between bacteria and carbohydrate in both the periods, as well as in pre-tsunami amino acid and bacteria has inversely correlated ($r = -0.223$). But an interesting positive correlation is observed between fungus and carbohydrate in both pre-and post-tsunami ($r = 0.777^{**}$ and $r = 0.630^{*}$).

Principal component analysis (PCA)

The evaluation of sediment nutrients (carbohydrate, amino acid) and microbial diversity (bacteria and fungus) relationship within the study area and the source identification, principal component analysis was used following standard procedure reported in literature (Jolliffe, 1986; Rubio et al., 2000; Yu et al., 2008; Dragović et al., 2008). PCA formed on the logarithmic form of the data variables. The results of the PCA for pre- and post-tsunami sediment samples are shown in Table 3 A and B. Three principal components (PCs) were extracted. Therefore, these three factors play a significant role in the microbial diversity in the study area. PCA was carried out using the PRIMER v6 software package (Plymouth Routines In Multivariate Ecological Research).

The Table 3 A and 3B illustrates that PC1 indicative of carbohydrate concomitant with amino acid and fungus. It may be due to the mixing of organic nutrients through riverine process. PC2 positively associated with bacteria. PC3 indicates a positive relationship with fungal diversity. The PCA showed that carbohydrate, amino acid and fungal diversity are strongly correlated in PC1. Bacterial diversity

Table 2A Pearson correlation matrix for pre-tsunami sediment samples

Parameter	Carbohydrate	Amino acids	Bacteria	Fungal
Carbohydrate	1.000	–	–	–
Amino acids	**0.910****	1.000	–	–
Bacteria	−0.163	0.011	1.000	–
Fungus	**0.630***	0.495	−0.193	1.000

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 2B Pearson correlation matrix for post tsunami sediment samples

Parameter	Carbohydrate	Amino acids	Bacteria	Fungal
Carbohydrate	1.000	–	–	–
Amino acids	−0.223	1.000	–	–
Bacteria	−0.509	0.135	1.000	–
Fungus	**0.777****	−0.213	−0.453	1.000

** Correlation is significant at the 0.01 level (2-tailed).

Table 3A Principal component analysis for pre tsunami sediment samples

Parameter	PC 1	PC 2	PC 3
Carbohydrate	**0.963**	0.074	−0.177
Amino acids	**0.902**	0.274	−0.291
Bacteria	−0.213	**0.958**	0.189
Fungus	**0.782**	−0.146	**0.605**

Table 3B Principal component analysis for post tsunami sediment samples

Parameter	PC 1	PC 2	PC 3
Carbohydrate	**0.903**	0.112	0.235
Amino acids	−0.385	**0.919**	0.089
Bacteria	−0.722	−0.220	**0.655**
Fungus	**0.881**	0.107	0.335

was remarkably dominant in PC2. In PC1 Bacterial diversity had a negative correlation to the other variables. In PC2 bacterial diversity had a positive correlation and carbohydrate and amino acid variables are also correlated with the PC2. But fungal diversity had a negative correlation to the other variables. In PC3 indicates a positive correlation in fungus and bacteria but the rest of them are negatively correlated.

Conclusion

The screening of tsunami sediments reveals that marine fungi and pathogenic microbial density are abundant in post tsunami sediments. The overall analysis indicates that the increase of pathogenic bacterial and fungal diversity in the post tsunami period is most probably due to inundation and the backwashing effect of the tsunami waves along the study area. The decrease in other bacterial diversity in the post tsunami sediments is most probably due to the demineralization of organic matter and continuous exchange of ocean water and backwashing of coastal sediments and it might be the retaining ability of the marine microbes in the intertidal ecosystem.

Acknowledgements

The authors would like to thank Prof. G. Victor Rajamanickam, former Dean of Tamil University, Tanjore, and SASTRA University for his kind assistance during the course of sample collection. The authors also thank Mrs. J. Elizabeth Christina, Assistant Professor, Scott Christian College, Nagercoil, Tamil Nadu, India, who helped in the proof correction of the manuscript. Finally, the authors thank Dr. Joseph Johnson, Director of Unity Foundation, and Tirunelveli, who helped to mould the manuscript in a spectacular way.

Cmpliance with ethics guidelines

Prince S. Godson, N. Chandrasekar, S. Krishna Kumar and Vimi P.V declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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